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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT :	Wands et al.		
SERIAL NUMBER :	09/436,184	EXAMINER :	K. Canella
FILING DATE :	November 8, 1999	ART UNIT :	1642
FOR :	DIAGNOSIS AND TREATMENT OF MALIGNANT NEOPLASMS		

November 22, 2004
Boston, Massachusetts

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R §1.132

I, Jack R. Wands, of East Greenwich, Rhode Island, declare and state as follows:

1. I am a co-inventor of the invention claimed in the above-referenced application and am employed by the named assignee, Rhode Island Hospital, Providence, Rhode Island.

2. I received a M.D. degree from the University of Washington in 1969 and currently serve as Chief of the Division of Gastroenterology at Lifespan Rhode Island Academic Medical Center, Director of the Liver Research Center, Professor of Medicine at Brown University School of Medicine, and Head of the Gastroenterology Section at Brown University. I am a member of the editorial boards of the academic journals Hepatology, International Hepatology Communications, Journal of Viral Hepatitis, and Viral Hepatitis Reviews, and serve as an editorial consultant for the Journal of Clinical Investigation, New England Journal of Medicine, Proceedings of the National Academy of Sciences, Journal of Infectious Disease, Gastroenterology, Journal of Virology, Virology and Nature Medicine. I have been involved in research relating to cancer for over 20 years.

3. I have read the Office Action mailed on May 21, 2004 and am familiar with the Examiner's grounds of rejection for lack of enablement of claims drawn to methods of inhibiting tumor growth using aspartyl (asparaginyl)-beta-hydroxylase (AAH) antisense nucleic acids.

4. Animal studies have been carried out to determine the effect of AAH antisense nucleic acids on tumors using a rat experimental model. Results indicated that AAH antisense oligonucleotides inhibited tumor growth and progression using a rat experimental model. The sequence of AAH antisense oligonucleotides was complementary to the sequence of human AAH (SEQ ID NO:3, shown on page 7 of the specification of the above-referenced patent application) and overlapped the initiation codon of the human AAH mRNA, starting at –1, -6, and –11 relative to the ATG initiating methionine-encoding codon of human AAH sequence, as shown below.

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5'CGGACCGTGC AATGGCCCAG CGTAAGAATG CCAAGAGCAG CGGCAACAGC AGCAGCAGCG  (SEQ ID NO:3)

3'GCCTGGCACG TTACCGGGTC GCATTCTTAC GGTTCACGTC GCCGTTGTCG TCGTCGTCGC 5'(COMPLEMENT)

            TTACCGGGTC GCATTCTTAC 5' (AAH antisense -1)
      GGCACG TTACCGGGTC GCATTCTT   5' (AAH antisense -6)
  GCCTGGCACG TTACCGGGTC            5' (AAH antisense -11)
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5. 9L gliosarcoma cells were contacted with AAH oligonucleotides or empty vector in vitro. The cells were incubated overnight. Rats were inoculated with 9L cells containing the AAH oligo; control rats received 9L cells containing empty vector. Mice were sacrificed daily from day 4 to day 10 of tumor inoculation, and the brains were harvested. A section through the site of inoculation was snap frozen for histologic analysis. In the control rats (empty vector), glioblastoma cells proliferated rapidly creating a large mass in the brain and invading brain tissue with fingerlike projections. In the AAH antisense group, tumor mass was substantially (50-75%) smaller compared to tumor mass in control rats, and fingerlike projections of tumor cells were not observed. Control rats became moribund within 18 days after tumor cell inoculation, whereas antisense-treated rats did not. As a result of the AAH antisense therapy, glioblastoma cells proliferated less rapidly in vivo, tumor masses were substantially reduced in size relative to control, and in some cases failed to grow.

6. The effect of the AAH oligonucleotides on infiltrative growth of other tumor cell types, cholangiocarcinomas and neuroblastomas, was tested in vitro. Three different human cholangiocarcinoma cell lines (originated from intrahepatic cholangiocarcinomas) were contacted with AAH antisense oligonucleotides described above. Negative controls were a sense AAH oligonucleotide or an oligonucleotide with an irrelevant sequence. Mean percentage of motile cholangiocarcinoma cells was reduced to 24% (cells transfected with antisense AAH oligonucleotides) from approximately 74% (cells transfected with sense AAH or an irrelevant sequence). AAH antisense oligonucleotides also significantly reduced mean percentages of motile neuroblastoma cells by 52% relative to data obtained using AAH sense oligonucleotides or nonrelevant oligonucleotides. The AAH antisense oligonucleotides inhibited AAH expression and infiltrative growth of tumor cells.

7. These data confirm that AAH antisense nucleic acids with a length of between 10-50 nucleotides, inclusive, and a sequence that is complementary to a 5' AAH regulatory sequence of SEQ ID NO:3 or a sequence that is complementary to a 5'coding region of SEQ ID NO:3 including the initiating ATG codon, inhibit tumor cell growth.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 11/22/04 J R Wands

Jack R. Wands

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